Production and characterization of a chimeric recombinant protein from synthetic genes consisting antigenic domain of EspA, Intimin and Tir of *Escherichia coli* O157 and evaluation as a candidate vaccine antigen in animal model

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Background: Enterohaemorrhagic *Escherichia coli* (EHEC) comprise an important group of zoonotic enteric pathogens. In humans, some EHEC infections result in bloody or non bloody diarrhea, which may be complicated by hemorrhagic colitis and severe renal and neurological sequelae, including hemolytic uraemic syndrome (HUS). Intimin, Tir, and EspA proteins are expressed by attaching-effacing Enterohemorrhagic *E. coli*. EspA proteins are part of the type three secretion system (TTSS) needle complexes that delivers Tir to the host epithelial cell, while surface arrayed intimin docks the bacterium to the translocated Tir. This intimate attachment leads to attaching and effacing (A/E) lesions. Vaccination strategies have been proposed for the prevention of this bacterium including the choice of potential antigens. EspA, intimin, and Tir are three predominant virulence factors of EHEC, and each of them has proved to be capable of inducing partial protective immunity.

Methods: In this study, we constructed a trivalent recombinant protein designated EIT that is composed of C-terminal 120 amino acids EspA (E), C-terminal 282 amino acids of intimin (I) and Tir fragment containing 103 amino acid residues 258 to 361 (T) which attached together using four repeat of five hydrophobic amino acids as a linkers. For high level expression of this chimeric gene in E.coli system the modified genes (1680 bp) encoding EspA, Intimin and Tir (EIT) was synthesized in accordance with the G + C content and increasing in the codon bias of *E. coli* genes without altering the encoded amino acid sequence. The multimeric effector was produced by using chemically induced T7 promoter in *E. coli* expression system. The immunization was assayed in mouse model with purified recombinant protein.

Results: The structure of synthetic gene, its mRNA and deduced protein and their stability was analyzed by bioinformatic softwares. Furthermore the immunogenicity of this multimeric recombinant protein consisting of three different domains was predicted. Experimental results showed that immunization with EIT induced strong humoral response to EspA, intimin and Tir and protected mice against the challenges with live EHEC.

Conclusion: This work suggests that for EHEC control a combination of antigenic EspA, Intimin and Tir appears to be more effective than using any of these immunogens alone.

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Variability of PspC (Pneumococcal surface protein C) in strains isolated in the University Hospital of the University of São Paulo (Brazil)

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Background: Streptococcus pneumoniae is one of the most common causes of respiratory tract infections. The vaccine composed of different capsular polysaccharides (PS) purified from pneumococci has low efficacy in children and the elderly, besides not being able to induce immunological memory. Although the 7-valent PS vaccine conjugated to CRM197 was an advance, the production cost is still a major barrier for its widespread use. A proposal to increase vaccine coverage at a low cost consists in the identification of protein antigens present in all pneumococcal isolates. PspC (Pneumococcal surface protein C) has been described for its role in both colonization of the nasopharynx and in invasive infection. PspC is highly polymorphic, being divided into 11 groups. Thus, the evaluation of the variability of this antigen in clinical samples is of great importance to determine the ideal vaccine formulation.

Methods: Pneumococcal strains were obtained from the University Hospital of the University of São Paulo (Brazil). Strains were serotyped by PCR and 13 isolates were chosen based on the serotypes present in the new 13-valent conjugate vaccine (serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F). The complete *pspC* locus was cloned and the gene was sequenced for each isolate. BALB/c mice were immunized with two different recombinant PspC variants for the production of antibodies that were used for Western blot analysis.

Results: From the 13 pneumococcal isolates analyzed, six were found to be from group 3, three isolates from group 6, one isolate from group 5, one isolate from group 8 and one isolate from group 9. A duplication containing PspC from group 4 and from group 10 was also found. An antiserum raised against PspC3 was able to recognize the majority of pneumococcal extracts in Western Blot analysis, showing a broad cross-reactivity. On the other hand, an antiserum raised against PspC8 was able to recognize only the isolate expressing PspC from group 8.

Conclusion: These preliminary results suggest that PspC3 would be a suitable vaccine antigen, being able to induce antibodies with broad cross-reactivity. Financial support: FAPESP, Fundação Butantan and CNPq.

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